

Multidrug-resistant *Escherichia coli* in African Catfish (*Clarias gariepinus*) Sold for Human Consumption in Maiduguri, Borno State, Nigeria: A Potential Public Health Concern

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ABSTRACT

The aim of this study was to determine the prevalence and antibiotic susceptibility profiles of *E. coli* in African catfish (*Clarias gariepinus*) in Maiduguri, Borno State, Nigeria. A total of 300 samples were collected from three major fish markets. *E. coli* was isolated and identified based on morphological and biochemical tests using standard bacteriological procedures. Confirmed *E. coli* isolates were screened for antibiotic susceptibility using the Kirby–Bauer disc diffusion method. The zones of inhibition were interpreted in accordance with Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing protocols. The overall prevalence of *E. coli* in African catfish was 14.0%. Location-specific prevalences of 12.0%, 14.0%, and 16.0% for the Tashan Bama Market, the Monday Market, and the Custom Market were detected, respectively. Skin samples had a significantly ($p = 0.046$) lower prevalence compared to intestinal samples. Almost all (97.6%) of the isolates were resistant to at least 2 or more different antibiotic agents, with the highest proportion (38.1%) being resistant to 7 antibiotic agents. Most of the isolates (61.9-92.9%) were resistant to pefloxacin, ampicillin, cefepime, tetracycline, augmentin, streptomycin and nalidixic acid. However, there was notable sensitivity to ofloxacin in several isolates (83.3%). Alarming, multidrug resistance was detected in 88.1% of isolates with an average MAR index of 0.62, indicating a high prevalence of antibiotic resistance among isolates recovered. To mitigate public health risk, strict hygienic practices in fish processing and marketing, public education on proper cooking, and the enforcement of rational antibiotic use in aquaculture are strongly recommended.

Keywords: Antimicrobial; Biochemical tests; *Escherichia coli*; Multidrug resistance; Prevalence

INTRODUCTION

Escherichia coli (*E. coli*) is a facultative Gram-negative bacterium commonly found in the intestines of warm-blooded animals and humans, serving as a key index of faecal contamination in water and food sources (Albuquerque, 2013; Nowicki *et al.*, 2021; Deblias *et al.*, 2025). While many strains of *E. coli* are harmless, certain pathogenic strains can cause severe gastrointestinal and extraintestinal infections in humans, ranging from diarrhoea to urinary tract, pulmonary and bloodstream infections and other illnesses (CDC, 2024). The spread of *E. coli* in aquatic environments, especially in freshwater fish, poses a significant public health concern due to the zoonotic potential of these bacteria and their ability to acquire and disseminate antibiotic resistance genes (Ndukwe and Ibrahim, 2024; Cheung *et al.*, 2025).

Freshwater fish are widely consumed in Nigeria, where fishing and fish marketing constitute vital socio-economic activities and sources of this major protein for the local population (Bradley *et al.*, 2020; Saba *et al.*, 2024). The high demand for fish, paired with often inadequate sanitary

conditions in fish harvesting, processing, and marketing, creates opportunities for bacterial contamination, including faecal indicator bacteria such as *E. coli* (Marijani, 2022; Marquis, 2022). Contamination can occur through several external factors like polluted water sources, unsanitary fish handling, and exposure to contaminated environments in markets and processing sites (Bibi *et al.*, 2015). The presence of *E. coli* in fish samples thus indicates potential health hazards for consumers, especially when fish are eaten without proper cooking or when the contaminated fish are handled improperly (Ali *et al.*, 2022).

Antibiotic resistance in *E. coli* isolated from freshwater fish is a growing global concern, complicating infection treatment and increasing transmission risks (Adenaya *et al.*, 2025). In Africa, extensive and often improper use of antibiotics in human health, veterinary practices, and aquaculture has significantly contributed to this challenge (Hassan *et al.*, 2021; Zhang *et al.*, 2022), a situation further exacerbated by inadequate regulation and monitoring,

particularly in countries like Nigeria (Ehsan, 2025). Aquatic environments, especially those impacted by untreated wastewater and agricultural runoff, serve as reservoirs for antibiotic-resistant bacteria and resistance genes (Titilawo *et al.*, 2015; Ndukwe and Ibrahim, 2024).

Widespread multidrug resistance (MDR) among aquatic *E. coli* isolates has been reported, with studies in Nigeria demonstrating resistance to commonly used antibiotics such as ampicillin, tetracycline, cotrimoxazole, gentamicin, penicillin, and erythromycin in both fish and water sources (Chigor *et al.*, 2010; Marijani *et al.*, 2022). This prevalence of MDR bacteria in fish destined for human consumption underscores the public health threat posed by persistent resistance within aquaculture environments.

Key risk factors for the presence and persistence of antibiotic-resistant *E. coli* in fish include environmental contamination from untreated wastewater, use of antibiotics in aquaculture, pond manuring, and poor hygiene practices throughout production and marketing (Dewi *et al.*, 2022; Adenaya *et al.*, 2025; Ibrahim *et al.*, 2024). Such conditions not only promote the development and spread of resistance but also increase the likelihood of cross-contamination during handling and storage. Consequently, resistant bacteria may be transmitted to humans via the food chain, water use, or direct contact, resulting in infections that are harder to treat; prolonged illness; increased healthcare costs; and greater risk of complications (Pelić *et al.*, 2024; Schmidt *et al.*, 2025).

Although a previous study conducted in the study area (Maiduguri) reported on MDR in fish (Grema *et al.*, 2015), the present study is crucial for tracking the spread of resistance in the local fish supply for human consumption. Therefore, the aim of this study was to determine the current occurrence of multidrug resistant *E. coli* in African catfish in Maiduguri, Borno State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in prominent fish markets across Maiduguri, the capital city of Borno State in Nigeria's Northeast sub-region. Maiduguri is located at 11°50' N and 13°09' E of the equator and has a population of approximately 1.9 million people, making it the largest city in Borno State. The state spans 69,435 square kilometres, covering about 7.69% of Nigeria's land. It shares borders with Niger, Chad, and Cameroon on the outside and Adamawa, Yobe, and Gombe states on the inside. Key water sources like Lake Alau, Lake Chad, and the Benue River support the region's fisheries and fish trade, making these markets ideal for study.

Study Design and Sampling Techniques

A cross-sectional study was conducted. Three major markets in the study area (Tashan Bama Market, Monday Market, and Custom Market) were selected purposefully based on their highest volume of trade. A total of 300 catfish samples were randomly purchased from different fish vendors in batches, 10 per purchase (i.e., 5 for skin and 5 for intestine) at an interval of five days for a period of thirty days between June and October 2025. At the market level, a systematic random sampling was employed in

selecting fish vendors to purchase fish for the study. The total number of vendors in the market was divided by the number of samples (n=10) required per day to obtain the sampling interval. Therefore, fish vendors to be sampled were selected at the end count of the fixed periodic interval.

Bacterial Isolation and Identification

Freshwater catfish skin surface and intestinal content were aseptically swabbed and transferred into sterile containers after appropriate labelling. Properly capped specimens were immediately placed in ice-packed boxes and transported to the Zoonoses and Food Safety Laboratory at the Department of Veterinary Public Health and Preventive Medicine, University of Maiduguri, and processed within 4 hours. In the laboratory, the specimens were homogenised and inoculated into nutrient broth for enrichment. The enriched broth culture was streaked on nutrient agar and incubated at 37°C for 24 hours under aerobic conditions for the development of typical greyish-white colonies. The colony was sub-cultured using the streak plate technique on Eosin Methylene Blue (EMB) agar and MacConkey agar plates repeatedly until the pure culture with clear colonial morphology was obtained. The isolates were also confirmed by their Gram staining reaction and biochemical tests such as the urease test, catalase test, indole test, citrate test, triple sugar iron test, Voges Proskauer test and Methyl Red test, as was described by Cheesbrough (2017).

Antibiotic Susceptibility Testing

Confirmed *E. coli* isolates were subjected to antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates following Clinical and Laboratory Standards Institute protocols (CLSI, 2024). A standardised inoculum (0.5 McFarland standard) was swabbed evenly across Mueller-Hinton agar plates. The concentrations of the antibiotics in the impregnated discs were as follows: ciprofloxacin (5 µg), ofloxacin (5 µg), pefloxacin (5 µg), nalidixic acid (30 µg), streptomycin (10 µg), tetracycline (30µg), ampicillin (10 µg), augmentin (20:10µg, amoxicillin: clavulanic acid), chloramphenicol (30 µg), and cefepime (30 µg), were placed on the agar surface using sterile forceps. The plates were then incubated at 35–37°C for 18–24 hours, after which the zones of inhibition were measured in millimetres with a calliper. The results were interpreted as susceptible, intermediate, or resistant where applicable using CLSI breakpoints except for pefloxacin for which EUCAST breakpoint was referenced as shown in Table 1.

Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotic resistance (MAR) index of each bacterial isolate was determined to evaluate the level of resistance to the antibiotics tested. The MAR index was determined by dividing the number of antibiotics that an isolate was resistant to by the total number of antibiotics that were tested against it. $MAR = a/b$

Where 'a' represents the number of antibiotics to which the isolate was resistant, and 'b' represents the total number of antibiotics tested. MAR index values greater than 0.2 were considered indicative of isolates originating from environments with high antibiotic exposure, while values of 0.2 or less suggested sources with lower antibiotic pressure (Krumperman, 1983).

Table 1. Antibiotic susceptibility breakpoints for *Enterobacteriales*.

Antibiotics	Susceptibility Breakpoints			Protocol
	Sensitive	Intermediate	Resistant	
Ofloxacin	≥ 16	13-15	≤ 12	CLSI
Pefloxacin	≥ 24	NA	< 24	EUCAST
Ciprofloxacin	≥ 26	22-25	≤ 21	CLSI
Augmentin	≥ 18	14-17	≤ 13	CLSI
Cefepime	≥ 25	NA	≤ 18	CLSI
Streptomycin	≥ 15	12-14	≤ 11	CLSI
Ampicillin	≥ 17	14-16	≤ 13	CLSI
Tetracycline	≥ 15	12-14	≤ 11	CLSI
Chloramphenicol	≥ 18	13-17	≤ 12	CLSI
Nalidixic acid	≥ 19	14-18	≤ 13	CLSI

EUCAST, European Committee on Antimicrobial Susceptibility Testing

Data Analysis

The data obtained from this study were analysed using SPSS version 20 (IBM, Armonk, NY: IBM Corp.). Positive results were calculated in proportion, and variables were assessed for evidence of association using chi-square reporting odds ratios and their respective 95% confidence intervals with Custom Market and intestines being the benchmarks for comparison. Any probability that is less than or equal to alpha (0.05) was considered statistically significant.

RESULT

Morphological and biochemical characteristics of isolates

Morphologically, when the isolates from the enriched broth culture were streaked on nutrient agar and incubated at 37°C for 24 hours, typical greyish-white colonies were observed. The isolates were found to be Gram-negative motile rods producing pink, round, moist lactose-fermenting colonies on McConkey agar and a green metallic sheen on EMB. Other characteristics are presented in Table 2.

Table 2. Morphological and biochemical characteristics of the bacterial Isolates.

Test Category	Characteristics/Test	Reaction
Morphological Characteristics	Appearance on MacConkey Agar	Pink, round, moist lactose-fermenting colonies
	Appearance on EMB Agar	Metallic green sheen colonies
Biochemical Characteristics	Gram Reaction	Gram-negative rods
	Indole	+
	Methyl Red	+
	Voges-Proskauer	-
	Citrate	-
	Catalase	+
	Urease	-
	TSI Reaction	A/A + Gas, No H ₂ S

+, Positive; -, Negative; A/A, Acid slant/Acid butt; H₂S, Hydrogen sulfide; TSI, Triple Sugar iron Test.

Prevalence of *E. coli* in catfish

Table 3 shows that out of the 300 fish samples, 42 were positive for *E. coli*, giving an overall prevalence of 14.0% (95% CI = 10.0, 19.7). In addition, Tashan Bama Market, Monday Market and Custom Market had 12.0%, 14.0% and 16.0% prevalence rates, respectively. However, in comparison with other markets, Custom Market had a higher likelihood of having positive results, and the differences were not statistically significant ($p > 0.05$). The results also demonstrated that skin samples (n = 150) had a significantly lower ($p = 0.046$) prevalence (10.0%) as compared to intestine samples (n = 150) with a prevalence of 18.0%.

Antibiotic susceptibility profiles exhibited by *E. coli* isolates from catfish in Maiduguri

Table 4 shows the result of antibiotic susceptibility testing for the 42 isolates to different antibiotics. Most of the isolates (61.9-92.9%) were resistant to pefloxacin, ampicillin, cefepime, tetracycline, augmentin, streptomycin and nalidixic acid. However, there was notable sensitivity to ofloxacin in several isolates (83.3%). Some isolates showed high intermediate sensitivity (warning signs) to certain antibiotics such as ciprofloxacin (59.5%) and chloramphenicol (38.1%). This pattern indicates a mixed antibiotic resistance profile among the *E. coli* isolates studied (Fig. 1).

Table 3. Prevalence of *E. coli* in skin and intestinal swabs of African catfish in Maiduguri.

Variable	Total No. Tested	No. Positive (%)	OR (95% CI)	Chi-square	P – value
Location					
Tashan Bama Market	100	12 (12.0)	0.72 (0.32-1.60)	0.664	0.415
Monday Market	100	14 (14.0)	0.86 (0.39-1.86)	0.157	0.692
Custom Market	100	16 (16.0)	Ref.	-	-
Tissue					
Skin	150	15 (10.0)	0.51 (0.26-1.00)	3.987	0.046
Intestine	150	27 (18.0)	Ref.	-	-
Overall	300	42 (14.0)	-	-	-

No., number; %, percent; OR, odd ratio; CI, confidence interval; NA, not applicable; Ref, reference category

Table 4. Antimicrobial susceptibility pattern of *E. coli* isolated from catfish in Maiduguri.

Antibiotics	No. (%)			
	Class	Sensitive	Intermediate	Resistant
Ampicillin	Beta-lactam	1 (2.4)	4 (9.5)	37 (88.1)
Tetracycline	Tetracycline	5 (11.9)	3 (7.1)	34 (81.0)
Streptomycin	Aminoglycoside	10 (23.8)	1 (2.4)	31 (73.8)
Ciprofloxacin	Fluoroquinolone	12 (28.6)	25 (59.5)	5 (11.9)
Pefloxacin	Fluoroquinolone	3 (7.1)	NA	39 (92.9)
Ofloxacin	Fluoroquinolone	35 (83.3)	6 (14.3)	1 (2.4)
Nalidixic acid	Quinolone	10 (23.8)	6 (14.3)	26 (61.9)
Cefepime	Beta-lactam	0 (0.0)	NA	36 (85.7)
Chloramphenicol	Amphenicol	15 (35.7)	16 (38.1)	11 (26.2)
Augmentin	Beta-lactam	7 (16.7)	1 (2.4)	34 (81.0)

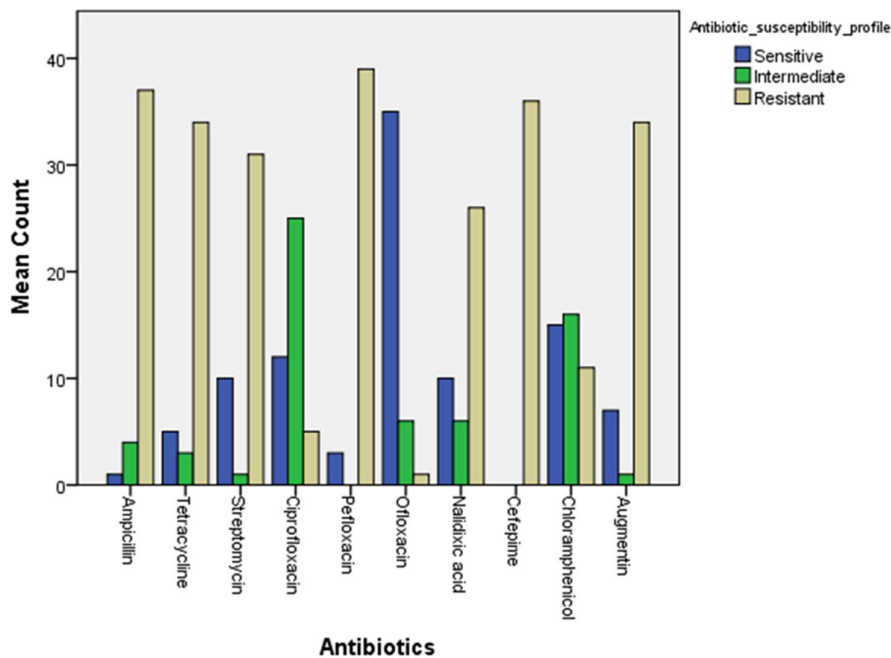


Figure 1: Showing patterns of antimicrobial susceptibility profile of the isolates

Multidrug resistance pattern of *E. coli* isolates from catfish in Maiduguri

Of the 42 isolates, 41 (97.6%) were resistant to at least two different antibiotic agents, as shown in Table 5. The highest proportion of *E. coli* isolates (38.1%) were resistant to 7 antibiotic agents, while none were resistant to 1 and 10 antibiotics. Overall, 37 (88.1%) isolates out of 42

were found to be resistant to at least one antimicrobial from three different classes of antimicrobials. The overall MAR index obtained for the isolates in this study was 0.62, indicating a high level of resistance to the antibiotics evaluated. The MAR value obtained exceeds the commonly accepted threshold of 0.2, suggesting that the isolates were likely derived from environments where antibiotics are frequently used or misused.

Table 5. Multidrug resistance pattern of *E. coli* isolated from catfish in Maiduguri

No. Antibiotic	Resistance to No. Antibiotics	
	No. Isolate	Proportion
0	1*	2.4*
1	0	0.0
2	3	7.1
3	4	9.5
4	2	4.8
5	3	7.1
6	2	4.8
7	16	38.1
8	10	23.8
9	1	2.4
10	0	0.0
Overall resistance	41	97.6%
MDR	37	88.1%

* , number and proportion sensitive to all 10 antibiotic agents; MDR=multidrug resistance- the number of isolates that demonstrated resistance to at least one antimicrobial in at least three classes of antimicrobials

DISCUSSION

The 14.0% prevalence of *E. coli* obtained in this study, with almost all isolates (97.6%) exhibiting resistance to antibiotic agents, is of serious public health concern because freshwater fish can harbour pathogenic and resistant strains of the bacteria (Bedane *et al.*, 2024; Alexandre *et al.*, 2025). The observed prevalence in this study is comparatively lower than those reported in Bangladesh (90.0%) and Egypt (92.7%), respectively, by Akter *et al.* (2022) and El-Badawy *et al.* (2025). Akande and Onyedibe (2019) also reported a slightly higher prevalence (17.5%) in Nigeria. These differences may be attributed to improved fish handling practices, lower environmental pollution in harvesting water and sampled markets, seasonal variations, or differences in sample size and methodology. However, the finding of this study was relatively higher than the previous study conducted in the study area by Grema *et al.* (2015).

In the present study, higher prevalence of *E. coli* was recorded in the intestine than in the skin, confirming the observation of Grema *et al.* (2015), who reported 50% prevalence in viscera compared with 34.6% in the skin. The difference was significant, and it could be aligned to the fact that *E. coli* resides in the intestinal tract, unlike other coliform bacteria that can appear in soil and water. Moreover, a higher intestinal load suggests that if fish are eviscerated (gutted) improperly in the market, the risk of cross contaminating the flesh and the environment increases significantly. Similarly, the distribution of *E. coli* also varied across market locations, with the prevalence more pronounced in Custom Market compared to the other markets (Custom Market > Monday Market > Tashan Bama Market). The differences could be attributed to levels of environmental contamination, because contaminated environments (markets) act as a melting pot for the exchange of resistance genes between different bacterial strains, as it was previously incriminated that poor sanitation and unhygienic market conditions are risk

factors for *E. coli* contamination in fish (Grema *et al.*, 2015; Akande and Onyedibe, 2019).

Despite antibiotic susceptibility testing revealing that ofloxacin currently works, the higher intermediate levels for its sister drug, ciprofloxacin, suggest that resistance is actively evolving in the Maiduguri environment. A similar scenario was observed for chloramphenicol; the higher intermediate sensitivity often indicates the beginning of the end for antibiotic efficacy. Notable resistance was observed against pefloxacin, ampicillin, cefepime, tetracycline, augmentin, streptomycin, and nalidixic acid. These results follow similar trends reported by Titilawo *et al.* (2015), Marijani *et al.* (2022) and Yohans *et al.* (2022), who reported high resistance to β -lactams and tetracyclines among aquatic *E. coli* isolates in Africa. The resistance observed may be attributed to overuse and misuse of antibiotics in aquaculture, cross-contamination from agricultural runoff, or antibiotic exposure through environmental sources, as emphasised by Agbabiaka *et al.* (2025). The presence of fluoroquinolone-sensitive strains suggests that these drugs remain effective for treating fish-associated *E. coli* infections; however, the resistance to commonly used antibiotics such as tetracycline, ampicillin, streptomycin and augmentin emphasizes growing antimicrobial resistance (AMR). The MAR index observed in this study is comparable to values reported in other aquaculture-related investigations. For instance, Sarter *et al.* (2007) reported MAR values ranging from 0.20 to 0.58 among Gram-negative bacteria isolated from farmed catfish, indicating considerable antibiotic exposure in aquaculture systems. Similarly, Akinbowale *et al.* (2006) documented MAR indices between 0.25 and 0.50 in bacteria isolated from aquaculture environments. In a related study involving freshwater fish, Hatha *et al.* (2005) reported MAR values ranging from 0.33 to 0.78, further demonstrating the widespread occurrence of antimicrobial resistance among bacteria associated with fish production systems.

Studies conducted within Nigeria have also highlighted the growing concern of antimicrobial resistance in aquatic and foodborne bacteria. For example, Adelowo *et al.* (2014) reported multidrug-resistant *E. coli* isolates from aquatic environments in southwestern Nigeria with MAR indices exceeding 0.2, suggesting significant antibiotic pressure in these ecosystems. The MAR value of 0.62 obtained in the present study therefore falls within the upper range of previously reported values and indicates a substantial burden of antibiotic resistance among *E. coli* isolates associated with catfish in the study area. Multidrug resistance noted in almost all isolates signals potential public health risks, as resistant bacteria may be transmitted to humans through handling or consumption of improperly cooked fish.

Conclusion

From the results obtained, it can be concluded that African catfish sold in Maiduguri markets harbour *E. coli*. The presence of *E. coli* on both the skin and intestines of fish indicates possible hygiene lapses during harvesting, transportation, processing, or marketing. The study also demonstrated that while most isolates remained susceptible to fluoroquinolones and chloramphenicol, considerable resistance existed against commonly used antibiotics, including ampicillin, tetracycline and augmentin. Multidrug resistance was detected in the majority of isolates with an average high MAR index, indicating a high prevalence of antibiotic resistance among isolates recovered. This reflects emerging AMR trends in environmental and food-borne bacteria. To mitigate public health risk, strict hygienic practices in fish processing and marketing, public education on proper cooking, and the enforcement of rational antibiotic use in aquaculture are strongly recommended.

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Conflict of Interest

The authors have no conflict of interest to declare.

Authors Contribution

AM, DJ and IYN contributed to the study concept and design. Material preparation, data collection and analysis were performed by AM, DJ, BM, SM, ASS, FAM, AIA and BG. The draft manuscript was prepared by AM, DJ, ZSN, and BG. AM, DJ, IYN, ASS and SM read, corrected and approved the manuscript for publication. All authors have read and approved the final manuscript.

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